

# Aspects of Protein Synthesis in Mengovirus-Infected Ehrlich Ascites Tumor Cells

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# Summary

In this thesis some aspects of the regulation of protein biosynthesis in Ehrlich ascites tumor cells after infection with mengovirus, a picornavirus, are discussed.

Chapter I describes the subject of this investigation and summarizes the design of the experiments which were performed to elucidate translational control after virus infection.

Chapter II is a compilation of the literature on the structure and infectious cycle of picornaviruses. Special attention is given to viral and host RNA and protein synthesis. The role of the cellular membranes in the biosynthesis of picornaviruses is discussed.

Chapter III - VIII consist of reports which have been published or are submitted for publication.

In Chapter III arguments are provided that postnuclear supernatants are a suitable system to study the relationship between host and viral protein synthesis in the infected cell. It contains a discussion of the similarities and differences of protein synthesis in mengovirus-infected cells and in postnuclear supernatants thereof.

Some differences between both systems are the consequence of changes in the intracellular concentrations of cations and ATP, between 3 hrs after infection and the end of the infectious cycle, 9 hrs after infection. Experiments dealing with this aspect of mengovirus infection are described and discussed in chapter IV.

Chapter V gives a more detailed account of the similarities between protein synthesis following mengovirus-infection, *in vivo* and *in vitro*. The synthesis of a less complex host protein fraction, the basic proteins, is examined. It is shown that the kinetics of shutoff of the synthesis of basic cellular proteins *in vivo* and *in vitro* after mengovirus infection are similar but not identical. The difference in rate constants of the inhibition is constant through the complete period of infection. Moreover, it is argued that several classes of host proteins exist, each with their own kinetics of shutoff.

In chapter VI, host mRNA isolated after virus infection is shown to be able to direct the synthesis of host-specific polypeptides in a cell-free protein-synthesizing system derived from uninfected cells. Host mRNA derived from virus-infected cells is as active as a template for cell-free protein synthesis as is host mRNA from uninfected cells. Therefore, shutoff of protein synthesis cannot be due to an increased nucleolytic degradation of pre-existing cellular mRNA.

In chapter VII, the development and characterization of homologous, fractionated, cell-free protein-synthesizing systems from mengovirus-infected and uninfected Ehrlich ascites tumor cells is described. These fractionated systems consist of ribosomes, an initiation factor fraction, a pH5 enzyme fraction, mRNA, and all other components required for protein synthesis: energy-rich compounds, an energy-regenerating system, amino acids and salts. The two systems show many similarities with respect to optimal levels of the

different components. They differ in the elongation rate of protein synthesis when programmed with a single mRNA species. Also, at supra-optimal concentrations of $Mg^{2+}$ and $K^{+}$, the system from virus-infected cells supports the translation of mengovirus RNA, but not that of host mRNA. Qualitatively and Quantitatively, the spectra of polypeptides synthesized in response to cellular mRNA are comparable in both systems. However, in a system derived from mengovirus-infected cells, three low-molecular weight products are synthesized in response to viral RNA, in addition to those viral polypeptides which are produced in a system from uninfected cells

Chapter VIII deals with the mechanism of selective translation of mengovirus RNA following infection of Ehrlich ascites tumor cells. Several components of the homologous, fractionated, cell-free protein-synthesizing systems derived from uninfected and mengovirus-infected cells were exchanged between both systems. Host and viral mRNA's were translated simultaneously. The selective translation of viral RNA is shown to reside mainly in the initiation factor fraction. The pH5 enzyme fraction from virus-infected cells is responsible for the remainder of the observed selectivity. However, host mRNA is also changed after virus infection. It is less able to compete with viral RNA during simultaneous translation than cellular mRNA from uninfected cells.

The results described in the experimental section of this study and their relation to recent reports in literature concerning the control of host and viral protein synthesis in picornavirus-infected cells are discussed in chapter IX. Our observations suffice to explain the selective translation of viral mRNA in the midphase and at the end of the infectious period. However, there is still no information on the events which occur immediately following penetration of the virus.